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Intraspecific variations and biological relationships of different populations of *Centaurea virgata* Lamark (Asteraceae) in Iran

Elaheh Ghasemi[1], Zeinab Toluei[1*]

[1]Department of Cell and Molecular Biology, Faculty of Chemistry, University of Kashan, P. O. Box: 87317-51167, Kashan, Iran.
[*]Corresponding author, Email: ztoluei@kashanu.ac.ir. Tel: +98-31-55912301.

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Abstract

Being a large genus in the Asteraceae family, the genus Centaurea is a complex and problematic taxon. *Centaurea virgata* Lamark is a widespread and invasive species of the Centaurea genus. This species is found in different regions of Iran. However, there are limited studies on this plant, especially at the morphologic and genetic level. The present study aimed to investigate the intraspecific variations of *C.virgata* collected from different regions of Iran based on morphological data. A morphometric study based on 27 quantitative and 28 qualitative morphological characters was carried out on 65 specimens from 31 populations. Cluster and principal coordinates analyses (PCO) of morphological characters showed that the 31 populations could be divided into three main groups and two subgroups. The analyses of variance (ANOVA) of morphological characters indicated that most of the evaluated characters were significantly different (P<0.05) among *C. virgata* populations. It also demonstrated that there were not significant differences (P<0.01) among populations of *C. virgata* for six morphological characters, including the width of the inflorescence leaves, inner phyllaries width, outer phyllaries width, outer phyllaries cilia length, phyllary rows number, and stamen number. It could be concluded that there is a high morphological variation among various populations of *C. virgata* collected from different regions of Iran. These morphological variations of *C. virgata* populations can be related to different ecological and environmental conditions or endogenous factors such as genetics, and the presence of adaptation mechanisms to specific situations.

Key words: Asteraceae, *Centaurea virgata*, Intraspecific variation, Morphology.

INTRODUCTION

Genetic diversity is the basis of corrective studies in many plant species and is a principal source of biodiversity (Hughes *et al.,* 2008). Fundamentally, the genetic resource is of immense importance in plant breeding, and henceforth, provides an opportunity for characteristic reproduction and generation (Weising *et al.,* 2005).

The study of genetic diversity between and within plant populations is performed in three different methods; namely, 1) morphological characteristics, 2) biochemical method, and 3) molecular markers (Semagn *et al.,* 2006). Morphological characteristics are the oldest and the most frequently used marker for the identification and classification of plants (Wang *et al.,* 2001). Prior to the use of molecular markers, scientists made use of the morphological characterizations to study the population structure and investigate the genetic diversity (Sattler and Rutishauser, 1997). The morphological method is studied per available and visible traits such as grain shape, flower color, growth behavior, and pigments. These features have many applications in plant descriptions and botanical keys and evaluation of genetic variation in the presence of environmental changes (Semagn *et al.,* 2006).

In different environments, variation in plants is usually considered as an adaptive mechanism, since plants respond to environmental changes by changing their morphological characteristics. Therefore, this can be concluded that species with greater morphological diversity are more adaptable to different environmental conditions. Studies on the morphological variations of the plants help us to understand the mechanism, manner, and effective factors of plant evolution and adaptation (Mal and Lovett-Doust, 2005).

The genus *Centaurea* L. (Asteraceae) includes about 800 species worldwide that are distributed in Asia, North America, and Europe (Mabberley, 1997; Hellwig, 2004).

Traditionally, many species of the genus Centaurea have been used to cure various ailments including diabetes, diarrhea, rheumatism, malaria, hypertension, etc. Moreover, chemical investigation on Centaurea clearly demonstrated that this genus mostly includes triterpenes, flavonoids, sesquiterpene, lignans, and lactones as the main characteristic of secondary metabolites (Aslan and Oksuz, 1999).

Centaurea virgata is a perennial plant, with a woody base and rose to purple flowers, which grows in different regions of Iran (Davis, 1970). In 1875, *C. virga*ta var. squarrosa (Willd.) Boiss. was introduced in the Flora of Orientalis (Boissier, 1875), and then, in 1908, its rank elevated to *C. virgata* subsp *squarrosa* (Willd.) Gugler (Guler and Halacsy, 1908). In Flora of Iran, *C. virgata* subsp. *squarrosa* has been reported and described (Mozaffarian *et al.,* 2019). Despite the wide distribution of this plant, there are only a few studies on the morphological variations of *C. virgata* species. Among the limited number of studies that have been carried out regarding this subject, one is "A taxonomic review of *Centaurea* L. sect. *Centaurea* (Asteraceae) in Iran" that includes *C. virgata* (Ranjbar and Negaresh, 2014). Also, *C. virgata* has been chemically studied (Tuzun *et al.,* 2017) with one study investigated *C. virgata* extract used in green synthesis of silver nanoparticles (AgNPs) and the isolated compound eupatorin (Tuzun *et al.,* 2018).

Morphological variations have been reported within and among some other species in the genus Centaurea (Vanderhoeven *et al*., 2002; Guarino and Rampone, 2006). The purpose of this study was to evaluate the variations in morphological characters of *C. virgata* populations from different regions of Iran for the first time.

MATERIALS AND METHODS

Plant materials

Sixty five specimens from 31 populations of *C. virgata* were collected from different regions of Iran from 2008 to 2018. Herbarium samples were prepared and deposited in the University of Kashan Herbarium (UKH). Information on understudied populations of *C.virgata* is listed in Table 1.

Morphological study

We analyzed morphological variation among the different populations of *C. virgata*. In total, 27 quantitative and 28 qualitative morphological characters were selected and evaluated (Tables 2 and 3). For statistical analyses, we encoded the qualitative characters as two or multistate data, and for the quantitative characters, standardized means were used in each individual. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check the normality of the quantitative data ($P<0.05$) (Appendix 1). Before analyzing the data, abnormal data were detected using MVSP software.

Statistical analysis

Cluster analysis and Principal Coordinates Analysis (PcoA) were performed using MVSP software Ver. 3.1 (Kovach, 1985). The hierarchical clustering algorithms were depicted with the UPGMA method and percent similarity coefficient for quantitative and qualitative characters (Gower, 1971; Podani, 1999). SPSS software Ver. 25 was used for comparison of means between groups. One-way ANOVA was performed for investigation of morphological variations among and within populations. The box plots were produced in order to illustrate the diagnostic characters. The Box plots provide a simple graphical distribution representation of the morphological data. The means of groups were compared using Tukey's test method. Finally, the Cophenetic coefficient was calculated with NTSYS 2.10e software (Rohlf, 2000).

RESULTS

Morphological variations between populations

Most of the populations showed significant differences ($P<0.05$) for the most morphological characters based on one-way ANOVA analysis. According to the obtained results, there was no significant difference ($P<0.05$) in characteristics such as inflorescence leaf width, phyllary rows number, outer phyllaries width, inner phyllaries width, median phyllaries cilia length, and stamen number (Appendix 2). Mean differences between the populations were significant in the case

Table 1. Voucher specimens of understudied populations of *C. virgata* from Iran.

No.	Locality	Individuals No.	Abbreviation	Altitude (m)	Herbarium No.
1	Luristan, Dorood, before Saravand village	2 (a, b)	dor121	2125	UKH*1021
2	Luristan, Nurabad	2 (a, b)	nur1031	1855	UKH 1031
3	Kashan, Ghohrud village	3 (a, b, c)	gho1050	2235	UKH 1050
4	Kashan, Alavi village	2 (a, b)	alv1052	1730	UKH 1052
5	Kashan, University of Kashan	2 (a, b)	uni1053	975	UKH 1053
6	Kashan, Eznaveh	3 (a, b,c)	ezn1054	2690	UKH 1054
7	Kashan, Barzok, Vishang	3 (a, b,c)	brz1058	2896	UKH 1058
8	Qazvin, Kouhin neck	2 (a, b)	qku1023	1563	UKH 1023
9	Qazvin, Avaj to Mahnian (Hamedan)	2 (a, b)	qav1048	2205	UKH 1048
10	Golestan, Maravehtapeh to Bojnourd , 35 km after Maravehtapeh	2 (a, b)	gol1025	1184	UKH 1025
11	Khorasan Razavi, Kalat to Mashhad	2 (a, b)	kal1028	1734	UKH 1028
12	Khorasan Razavi, Neyshabur to Kashmar, 10 km to Rivash, 15 km to Kashmar	2 (a, b)	ney1029	2032	UKH 1029
13	North Khorasan, Shirvan, Kouseh bifurcate	2 (a, b)	shi1027	1697	UKH 1027
14	North Khorasan, Esfarayen to Bojnurd, Asadli neck	2 (a, b)	esf1030	1718	UKH 1030
15	Kurdistan, Marivan to Saqqez, 65 km after Marivan, between Aqjeh and Qamjian	2 (a, b)	mrv1032	1799	UKH 1032
16	Kurdistan, Bijar, Khosroabad	2 (a, b)	bik1033	1710	UKH 1033
17	Kurdistan, Bijar to Zanjan, Shirinbolagh bifurcate	2 (a, b)	biz1034	1626	UKH 1034
18	Kurdistan, Sanandaj to Divandareh, 30 km to Divandareh, before AqBolagh village	2 (a, b)	san1037	1987	UKH 1037
19	Zanjan, Mahneshan, bifurcate of Hasanabad and Hussainabad villages	2 (a, b)	mah1035	2083	UKH 1035
20	Zanjan, Mahneshan to Halab	2 (a, b)	hal1036	1772	UKH 1036
21	West Azerbaijan, Bukan to Mahabad, 25 km to Mahabad	2 (a, b)	buk1038	1805	UKH 1038
22	West Azerbaijan, Oshnavieh to Orumieh, 5 km after AqBolagh village	2 (a, b)	osh1039	2181	UKH 1039
23	West Azerbaijan, Oshnavieh to Orumieh, 2-3 km to Jarabad village	2 (a, b)	osh1040	1828	UKH 1040
24	West Azerbaijan, Orumieh, after Silvaneh, after Toly village	2 (a, b)	oru1041	1665	UKH 1041
25	West Azerbaijan, Orumieh, Movana to Neychalan	2 (a, b)	oru1043	1694	UKH 1043
26	West Azerbaijan, Orumieh, Movana to Neychalan	2 (a, b)	oru1044	1715	UKH 1044
27	West Azerbaijan , before Chaldoran, Alimardan village	2 (a, b)	chl1045	2005	UKH 1045
28	West Azerbaijan, Maku, Baduli village	2 (a, b)	mal1047	1929	UKH 1047
29	Tehran, 5 km after Polur-Firoozkooh bifurcate	2 (a, b)	teh1024	2271	UKH 1024
30	Hamedan, Malayer	2 (a, b)	ham1061	1813	UKH 1061
31	Hamedan, Malayer	2 (a, b)	ham1062	1794	UKH 1062

*University of Kashan Herbarium.

of traits, e.g. the highest flowering axis length was observed in Zanjan, Mahneshan (1035) (54 cm) which did not have a statistically significant difference with Kurdistan, (1032), Zanjan, (1036), West Azerbaijan, (1039) and Hamedan (1062) (43, 40, 41, 40.5 cm respectively) showed significant differences with other populations. The highest involucres length was observed in North Khorasan, population (1030) (10 mm), which showed statistically significant difference with all populations, and the highest involucres width was observed in North Khorasan, (1030) and Khorasan Razavi (1029) populations (5 mm).

Table 2. List of quantitative characters used in morphological studies.

No.	Characters	Unit of measurement	No.	Characters	Unit of measurement
1	Plant length	cm	15	Flowering axis length	cm
2	Inflorescence leaf length	mm	16	Inflorescence leaf width	mm
3	Median leaf length	cm	17	Median leaf width	mm
4	Median leaf segment length	mm	18	Median leaf segment width	mm
5	Median leaves segment number		19	Upper leaf length	cm
6	Upper leaf width	mm	20	Capitula pedicle length	cm
7	Involucres length	mm	21	Involucres width	mm
8	Phyllary rows number		22	Outer phyllaries cilia number	
9	Outer phyllaries length	mm	23	Outer phyllaries width	mm
10	Outer phyllaries cilia length	mm	24	Inner phyllaries width	mm
11	Median phyllaries cilia number		25	Inner phyllaries length	mm
12	Median phyllaries length	mm	26	Stamen number	
13	Median phyllaries width	mm	27	Median phyllaries cilia length	mm
14	Segment number of median leaf				

Table 3. List of qualitative characters used in morphological studies.

No.	Characters	Numerical code
1	Stem hair type	0- Arachnoid*
2	Stem hair density	0- Loose 1-Dense 2- More in down part
3	Inflorescence leaf hair type	0- Arachnoid
4	Inflorescence leaf hair density	0- Low 1-Loose 2- Dense
5	Median leaves hair type	0- Arachnoid
6	Median leaf margin shape	0- Entire
7	Median leaf apex shape	0- Acute
8	Upper leaves hair type	0- Arachnoid
9	Upper leaves hair density	0- Low 1-Loose 2- Dense
10	Upper leaf margin shape	0- Entire
11	Upper leaf apex shape	0- Acute
12	Inflorescence type	0- Cyme
13	Flowering axis flower number	1- Single 2- Single and two 3- Single and four
14	Involucres shape	0- Ovate 1- Fusiform
15	Outer phyllaries shape	0- Ovate
16	Outer phyllaries hair shape	0- Linear
17	Outer phyllaries hair density	0- Low 1- Loose
18	Outer phyllaries appendage shape	0- Cilia and spin 1-Spin
19	Inner phyllaries hair type	0- Glabr
20	Median phyllaries shape	1- Fusiform
21	Median phyllaries hair shape	0- Linear
22	Median phyllaries hair density	0- Low
23	Median phyllaries appendages shape	0- Cilia, spin 1- Cilia, non spin
24	Petal apex shape	0- Lanceolate
25	Stamen cilia	0- Cilia 1- Non cilia
26	Upper leaf type	0- Simple 1- Bipinnatipartite 2- Threepinnatipartite
27	Anther shape	0- Linear
28	Stem branch condition	0- Non branched 1- Branched at base

*Characters that have only one code are the same in all populations.

The highest Median phyllaries length was observed in the population of Hamedan (1061) (7.5 mm), which showed a significant difference with all populations (Appendix 3).

Cluster analysis

Three main groups and two subgroups were detected in the dendrogram resulted from cluster analysis of 55 quantitative and qualitative characters (Figure 1): Group A consisted of North Khorasan (1030), Khorasan Razavi (1029), Kurdistan (1032 and Qazvin (1048) populations.

West Azerbaijan (1047, 1045, 1043, 1040), Kurdistan (1037, 1034) and Khorasan Razavi (1028) fell into group B and the rest of the populations were categorized in group C. Then, group C was divided into two subgroups C1 and C2; subgroup C1 comprised of the West Azerbaijan population (1038), all the populations of Kashan (1050, 1052, 1054, 1058), and all the populations of Hamedan (1061, 1062). Subgroup C2 included all the populations of Luristan (1021, 1031), all the populations of Zanjan (1035,1036), all the individuals of Golestan population (1025), Qazvin (1023), Tehran (1024), North Khorasan (1027), Kurdistan (1033) and West Azerbaijan (1044, 1039).

Distribution of different populations of *C. virgata* by PcoA (Figure 2), confirmed the results of cluster analysis. Eigenvalues, individual percentages, and cumulative percentages of variance among the data accounted for the first three axes of the PCO are presented in Table 4. Box plots of the diagnostic morphological characters in different groups have been shown in Figures 3 and 4. According to the Box plots, group I was separated from the other groups by maximum median leaf segment width, maximum capitula pedicle length, involucres length, involucres width, flowering axis flower number, and minimum inflorescence leaf hair density. Group II was separated by minimum flowering axis length, median leaf length, median phyllaries length, and group III was separated from the two other groups by minimum stem hair density. The group III was divided into two subgroups, which were separated from each other by inner phyllaries length, outer phyllaries length, median

Table 4. Eigenvalues, individual and cumulative percentage variance of populations data accounted for the first three axes of the Principal Coordinates Analysis (PCO) for the *C. virgata* from Iran.

Initial eigenvalues	Axis 1	Axis 2	Axis 3
Eigenvalues	62.738	43.463	30.777
Percentage	27.335	18.936	13.403
Cum. percentage	27.335	46.271	59.680

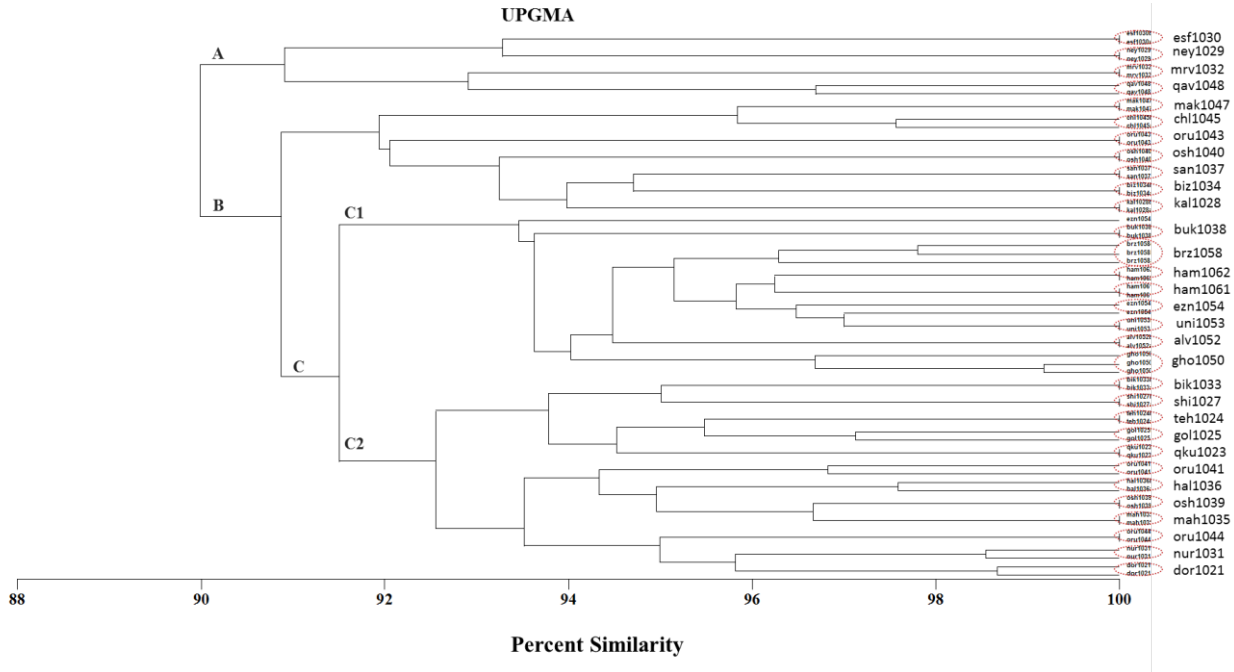

Figure 1. The dendrogram based on the analyses of morphological data in 31 populations (65 specimens) of *C. virgata* from different regions of Iran. Three main groups (A, B and C) and two sub groups (C1 and C2) can be distinguished.

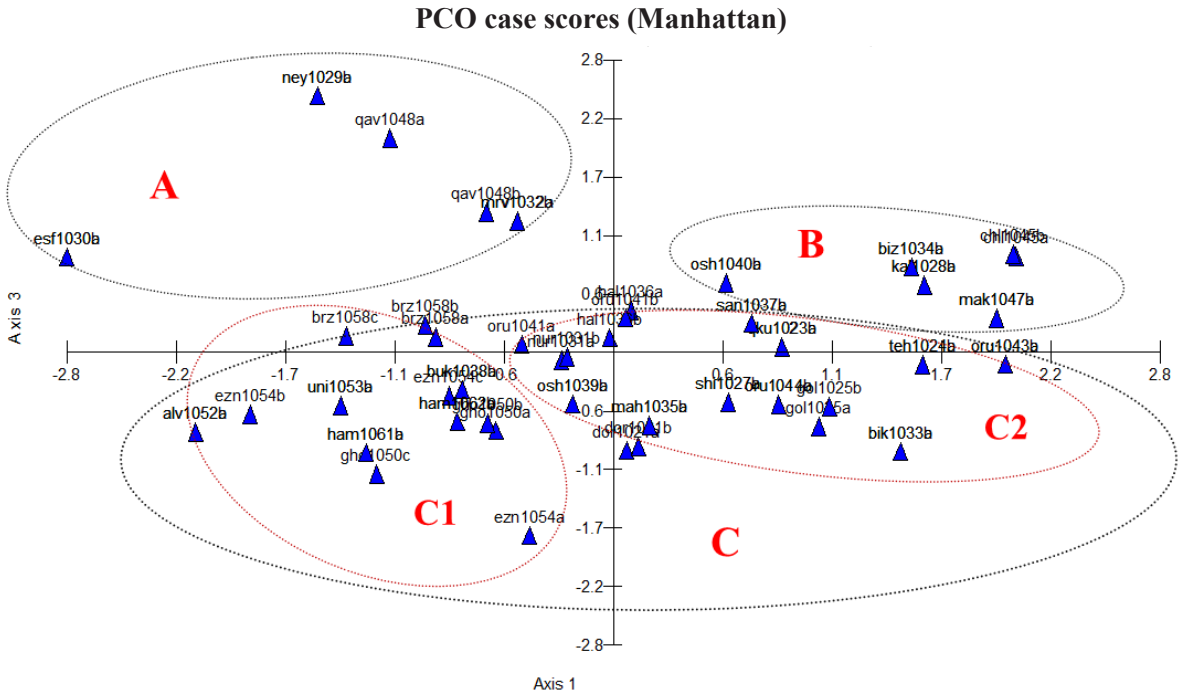

Figure 2. PCO of 31 populations (65 specimens) of *C. virgata* in Iran based on morphological data, populations are summarized into three main groups (A, B, C) and two subgroups (C1 and C2).

leaf segment length, median leaf segment width and inflorescence leaf length. The cophenetic coefficient was 0.982. So it could be said that clustering was fit.

DISCUSSION

Intraspecific variations of *C. virgata* were investigated in the present study. This is noteworthy that there is a high morphological variation among various populations of *C. virgata* collected from different regions of Iran. Analysis of variance on morphological characters showed that most of the evaluated characters were significantly different among populations ($p \leq 0.05$). The results of our study have also shown three distinct main groups and two subgroups. With reference to these groups, there is a slight consistency between the morphological clustering and geographical distribution in populations such as Kashan, Luristan, Golestan, Hamedan, and Zanjan. Although most of the populations of West Azerbaijan were placed in one group, some other populations from this region were placed in other groups. Although the populations of some provinces such as Khorasan Razavi were geographically close to each other, they were morphologically distinct from one another. This explains that geographic position is not the cause of morphological variations of these populations. Hence, separation of these populations can be related to different ecological and environmental conditions or internal factors such as genetics, and the presence of adaptation mechanisms to specific situations. The clustering of our understudied populations can be related to chromosome numbers and ploidy levels. Further studies must be undertaken to distinguish the three groups as cytotypes, chemotypes, and varieties on *C. virgata*. Regarding other species of the genus Centaurea, there have been other studies including the research work done by Mráz *et al.* (2011) who studied more than 40 morphological characters on 78 populations of *Centaurea stoebe* L., which were grown under greenhouse conditions. Morphological analyses demonstrated a clear separation of 2x and 4x plants and, thus, supported the diagnosis of both cytotypes as separate taxa. Differences in the number of florets, the life cycle, the shape of capitula, and the shape of young rosette leaves were the important discriminating traits. Besides, karyological and multivariate morphometric analyses of *Centaurea stoebe* were used to demonstrate the morphological differentiation and their cytotype distribution patterns. The morphological tendency towards cytotype differentiation is evident only at a population level (Spaniel *et al.,* 2008). *Centaurea phrygia* agg. was studied to investigate its morphological and ploidy level variation. Thriploid levels were found and morphometrical analysis demonstrated the separation of the taxa; the length

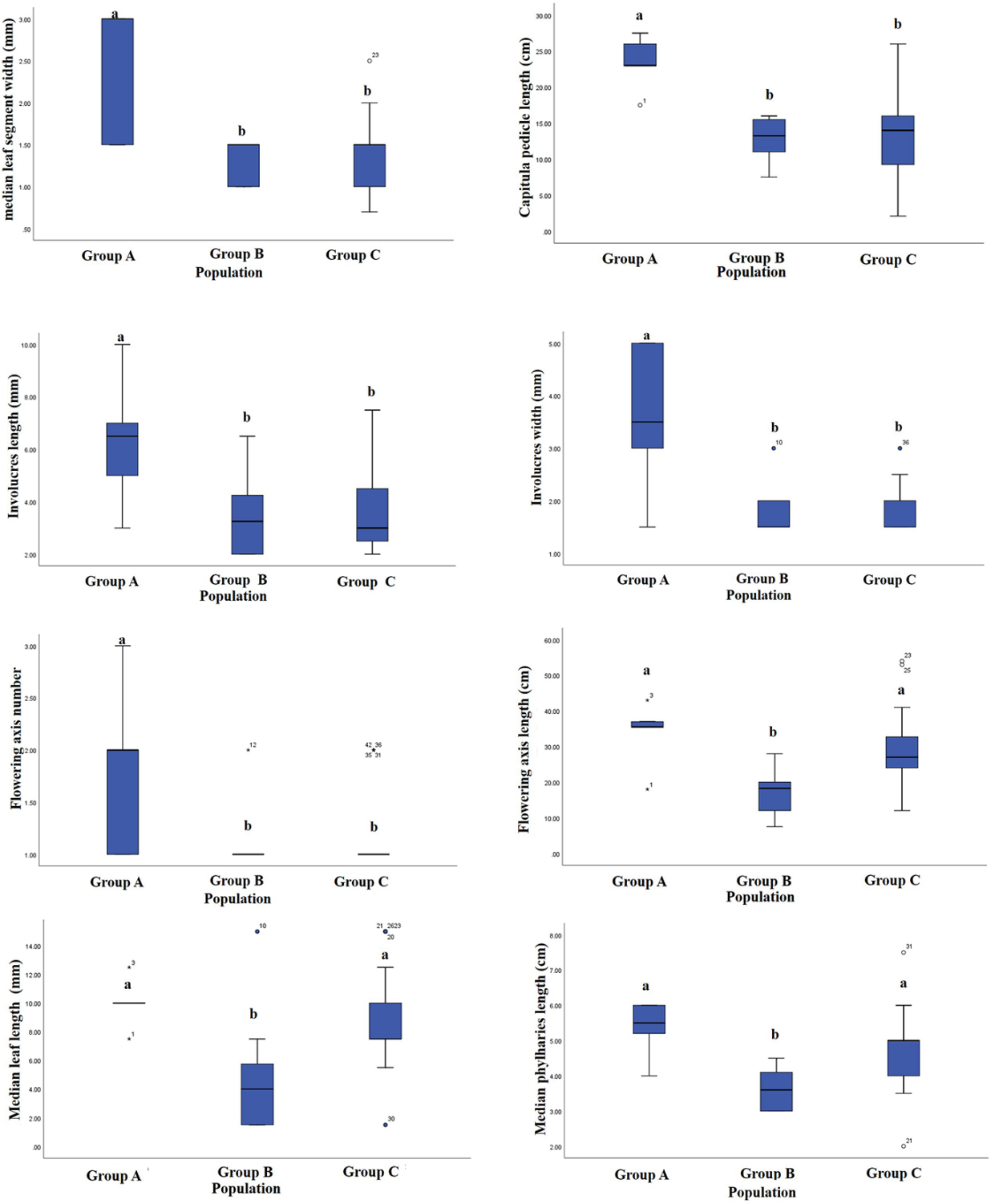

Figure 3. Box plots of diagnostic morphological characters in different populations of *C. virgata* among groups analyzed by SPSS software.

and the width of an involucre, visibility of appendages of inner involucral bracts, the length and the width of appendages of middle involucral bracts, and the length/width ratio of middle cauline leaves are the most important discriminant characters (Koutecky, 2007). According to Shabestari *et al.* (2013) on seed morphological traits and seed surface ornamentations in nine *Centaurea* species, the UPGMA clustering based on the morphological characters divided the species into distinct groups. Moreover, the ANOVA

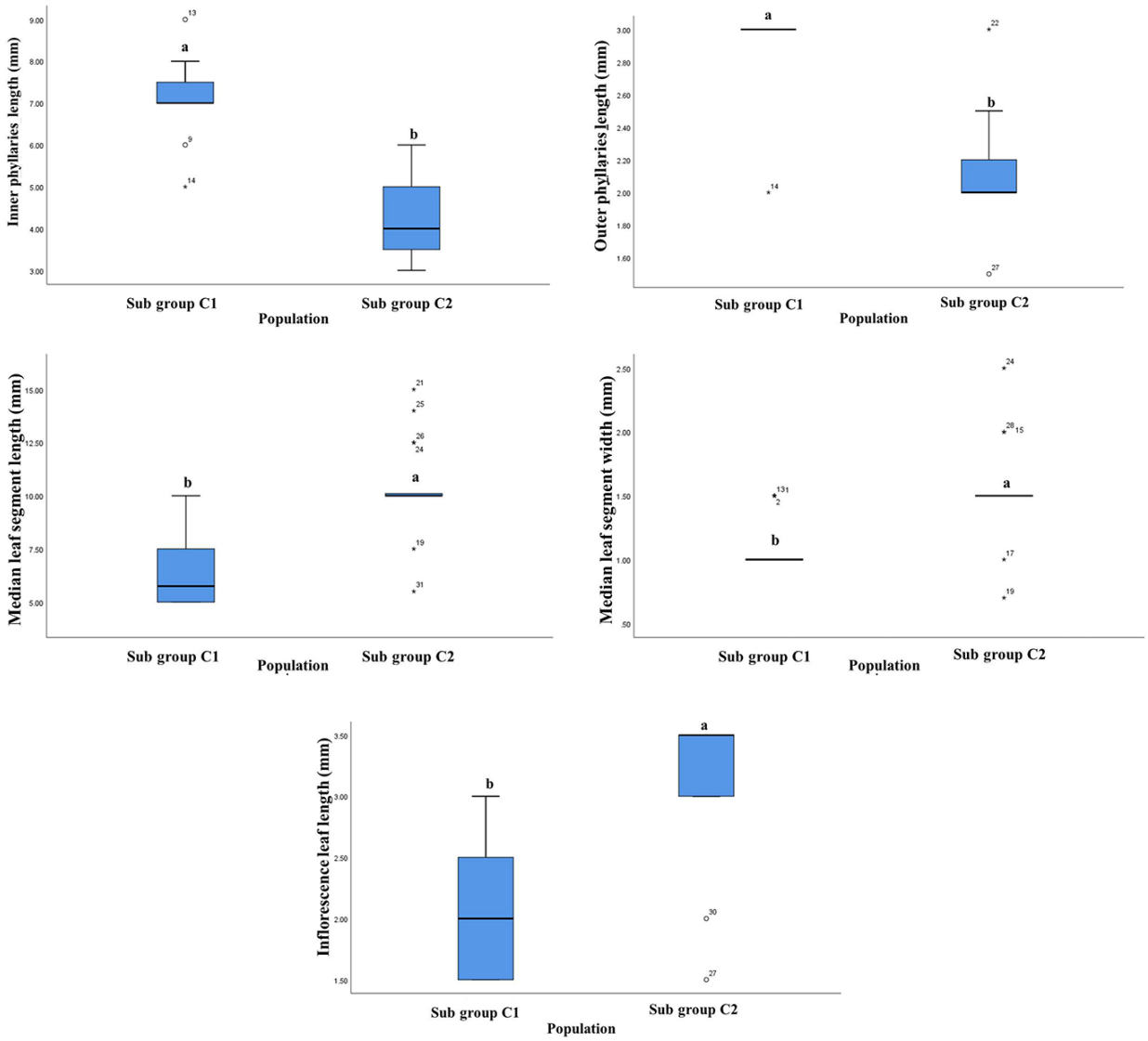

Figure 4. Box plots of diagnostic morphological characters among subgroups C1 and C2 analyzed by SPSS software.

test performed on nine quantitative seed morphological traits revealed a significant difference among the studied species ($p \leq 0.05$). Multivariate morphometric analysis on taxa of Jacea-Lepteranthus (*Centaurea* group) showed significant differences between species and some hybrids in several morphological characters (length of appendages on middle involucral bracts, fimbria length, length of the involucre, and the ratio between length and width of the appendage of middle bracts) (Vonica *et al*., 2013). As a result, based on our study and the other similar studies on the genus *Centaurea*, it is clear that morphological characters are discriminative and useful markers for the differentiation of various species of this genus. Two mechanisms are expressed for the ability to disperse populations against changes of the habitat, phenotypic plasticity and adaptation. Phenotypic plasticity is the capacity of a single genotype to produce different phenotypes in response to environmental changes, therefore, phenotyping is not only the occurrence of genotypes; rather it is influenced by environmental factors that play an important role in changing genotypes and phenotypes (Sexton and Mckay, 2002). Phenotypic plasticity might affect evolution in two ways: (1) by the process of genetic accommodation, whereby natural selection acts to improve the regulation, form, and phenotypic integration of novel phenotypic variants, and (2) through stimulating evolutionary responses to environmental changes via population persistence (Schlichting and Wund, 2014).

The result of our study on this species showed the existence of the variation at the phenotypic level. Con-

sequently, further studies are needed in order to determine the level and type of genetic diversity).

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REFERENCES

Aslan U., and Oksuz S. (1999). Chemical constituents of *Centaurea cuneifolia*. *Turkish Journal of Chemistry*, 23(1): 15–20.

Boissier P. E. (1875). *Centaurea virgata* var. *Squarrosa*. In Boissier E. (Ed.), Georg H., Geneva & Basilea [Basel], Flora of Orientalis, 3: 651.

Davis P. H. (1970). Flora of Turkey and the East Aegean Islands. (3rd Ed.), Edinburgh University Press, Edinburgh.

Gower J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, 27(4): 857–871.

Guarino C., and Rampone S. (2006). A morphometric analysis of *Centaurea* sect. *Dissectae* (Compositae). *Bocconea*, 19: 77–88.

Guler W., and Halacsy E. V. (1908). Die bisher bekannten *Centaurea*-Arten Griechenlands. *Annales Historico-Naturales Musei Nationalis Hungarici*, 6: 15–297.

Hellwig F. H. (2004). Centaureinae (Asteraceae) in the Mediterranean history of ecogeographical radiation. *Plant Systematics and Evolution*, 246(3-4): 137–162.

Hughes A. R., Inouye B. D., Johnson M. T., Underwood N., and Vellend M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, 11(6): 609–623.

Koutecky P. (2007). Morphological and ploidy level variation of *Centaurea phrygia agg.*(Asteraceae) in the Czech Republic, Slovakia and Ukraine. *Folia Geobotanica*, 42(1): 77–102.

Kovach W. (1985). Institute of Earth Studies, University College of Wales, ABERYSTWYTH, (Shareware), MVSP Version 3.2, 1985-2002 Kovach Computing Services. [Online] Available: *http://www. kovcomp. com/ MVPs/downl2. Html* (2002).

Mabberley D. J. (1997). The plant-book: a portable dictionary of the vascular plants. Cambridge University Press.

Mal T. K., and Lovett-Doust J. (2005). Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, Lythrum salicaria (Lythraceae), in response to soil moisture. *American Journal of Botany*, 92(5): 819–825.

Mozaffarian V., Ghahramaninejad F., Narimisa S., Jafari E., Osalu Sh. K., Lotfi E., and Asadi M. (2019). Family Asteraceae. In Assadi M., Masoumi A. A., and Mozaffarian V. (Eds.), Tehran: Research Institue of Forests and Rangelands, Flora of Iran, 144: 304–308.

Mráz P., Bourchier R. S., Treier U. A., Schaffner U., and Müller-Schärer H. (2011). Polyploidy in phenotypic space and invasion context: a morphometric study of *Centaurea stoebe* sl. *International Journal of Plant Sciences*, 172(3): 386–402.

Podani J. (1999). Extending Gower's general coefficient of similarity to ordinal characters. *Taxon,* 48(2): 331–340.

Ranjbar M., and Negaresh K. (2014). A revision of *Centaurea* sect. *Centaurea* (Asteraceae) from Iran. *Turkish Journal of Botany*, 38: 969–987.

Rohlf F. J. (2000). NTSYS-pc: numerical taxonomy and multivariate analysis system. Version 2.10e. Exeter Ltd., Setauket.

Sattler R., and Rutishauser R. (1997). The fundamental relevance of morphology and morphogenesis to plant research. *Annals of Botany*, 80(5): 571–582.

Schlichting, C. D., and Wund M. A. (2014). Phenotypic plasticity and epigenetic marking: an assessment of evidence for genetic accommodation. *Evolution*, 68(3): 656–672.

Semagn K., Bjornstad A., and Ndjiondjop M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, 5(25): 2540–2568.

Sexton J. P., Mckay J. K., and Sala A. (2002). Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Application*, 12(6): 1652–1660.

Shabestari E. S. B., Attar F., Riahi H., and Sheidai M. (2013). Seed morphology of the *Centaurea* species (Asteraceae) in Iran. *Phytologia Balcanica*, 19: 209–214.

Spaniel S., Marhold K., Hodalova I., and Lihova J. (2008). Diploid and tetraploid cytotypes of *Centaurea stoebe* (Asteraceae) in Central Europe: morphological differentiation and cytotype distribution patterns. *Folia Geobotanica*, 43(2): 131–158.

Tuzun B. S., Hohmann J., and Kivcak B. (2018). Green bio-inspired synthesis, characterization and activity of silver nanoparticle forms of *Centaurea virgata* Lam. and the isolated flavonoid eupatorin. *Green Processing and Synthesis*, 7(4): 372–379.

Tuzun B., Hajdu Z., Orban-Gyapai O. P., Zomborszki Z., Jedlinszki N., Forgo P., and Hohmann J. (2017). Isolation of chemical constituents of *Centaurea virgata* Lam. aAnd xanthine oxidase inhibitory activity of the plant extract and compounds. *Medicinal Chemistry*, 13(5): 498–502.

Vanderhoeven S., Hardy O., Vekemans X., Lefebvre C., De Loose M., Lambinon J., and Meerts P. (2002). A morphometric study of populations of the *Centaurea jacea* complex (Asteraceae) in Belgium. *Plant Biology*, 4(03): 403–412.

Vonica G., Frink J. P., and Cantor M. (2013). Taxonomic revision of some taxa of Jacea-Lepteranthus group (*Centaurea* genus) based on morphometric analysis. *Brukenthal Acta Musei*,584–567 :(3)8 .

Wang Y. F., Ferguson D. K., Zetter R., Denk T., and Garfi G. (2001). Leaf architecture and epidermal characters in *Zelkova*, Ulmaceae. *Botanical Journal of the Linnean Society*, 136(3): 255–265.

Weising K., Nybom H., Pfenninger M., Wolff K., and Kahl G. (2005). DNA fingerprinting in plants: principles methods and applications. CRC Press.